

# Supplementary Figure 1: Characterisation of the incompatible interaction in *C. grandiflora* x *C. rubella* hybrids.

**a** Results of two-dimensional QTL mapping for the incompatible phenotype. The full LOD score is shown below the diagonal, the interaction LOD score above. Colour scale refers to interaction LOD

score (left) and full LOD score (right). This QTL experiment has been performed using the phenotypic average values of 10 replicates for 142 RILs.

**b** Phenotypes of NIL plants with the indicated genotypes. Yellow font indicates incompatible phenotypes.

**c,d** Leaf area (**c**) and leaf-cell area (**d**) in the indicated genotypes. Values are mean  $\pm$  s.e.m. of 10 and 4 leaves respectively per genotype, respectively. Letters indicate significant differences as determined by Tukey's HSD test ( $\alpha = 0.05$ ).



# Supplementary Figure 2: Characterization of the constitutive immune response in incompatible hybrids.

**a** Expression of the indicated immune-response markers determined by qRT-PCR normalized to *Capsella TUB6*. Mean  $\pm$  s.e.m. of three biological replicates is shown. Letters indicate significant differences as determined by Tukey's HSD test ( $\alpha = 0.05$ ).

**b** Trypan Blue staining of leaves from the indicated genotype fails to detect ectopic necrosis in incompatible hybrids. Positive control is an early senescent leaf.

**c** The incompatible phenotype in NIL(2gg; 7rr) plants (right) can be rescued by growth at elevated temperatures as compared to compatible control plants (left).



# Supplementary Figure 3: Fine-mapping of the incompatible loci and transgenic rescue of the incompatible phenotype.

**a**, **b** Location of markers used for mapping (top), genotypes and associated phenotypes of informative recombinants (middle) and annotated genome structure in the QTL2 (**a**) and QTL7 regions (**b**). Rectangles in the genome annotation represent exons, solid lines are introns and dotted lines show intergenic sequences.

c Leaf size in the indicated genotypes. Values are means  $\pm$  s.e.m. from 4 leaves per genotypes. Numbers indicated independent transgenic lines for the same construct. Letters indicate significant differences as determined by Tukey's HSD test ( $\alpha = 0.05$ ).

**d-k** Expression of *NPR1* (**d**,**g**), *RPP5* (**e**,**h**) and its closest *Capsella* paralogue *RPP5h* (**f**), and of immune-response markers *EDS1* (**i**), *EDS5* (**j**) and *PDF1.2* (**k**) determined by qRT-PCR normalized to *Capsella TUB6*. Mean  $\pm$  s.e.m. of three (**d-f**) or four (**g-k**) biological replicates is shown. Letters indicate significant differences as determined by Tukey's HSD test ( $\alpha = 0.05$ ). Numbers indicated independent transgenic lines for the same construct as in (**c**).

100 Cg926 NPR1 Co NPR1 Cr1504 NPR1 100 Aly NPR1 100 Aly NPR1 100 Aly NPR1 99 BraraA03 NPR1 BraraA03 NPR1 BraraA01 NPR1

F

0.05

| b  | 10 20  | 20 40  | 50 60   | 70 80 80  | 100 110 120 120  |
|--|--|--|---|---|--|
| Cr1504_NPR1<br>Cg926_NPR1<br>Co_NPR1<br>Np_NPR1<br>Aly_NPR1<br>At_NPR1<br>ThA1_NPR1<br>BraraA03_Npr1<br>BraraA06_NPR1<br>BraraA01_NPR1     | MDTNUDGF/DSYBISS      ASFFAA         MDTNUDGF/DSYBISS      ASFFAA  | APAFTESSIVYPAAEKL-ITG<br>  | POVSALRILS STESVEDSPE   | D FYSDAKLVLAD CREVSFHC VVSARS<br>D FYSDAKLVLAD CREVSFHC VVSARS<br>D   | IO         IO         IO         IO         IO           FFKNALAABE-KEKDSN-AUVELKSIAKOYS   |
| Cr1504_NPR1<br>Cg926_NPR1<br>Co_NPR1<br>Np_NPR1<br>Aly_NPR1<br>At_NPR1<br>Thal_NPR1<br>BraraA03_Npr1<br>BraraA08_NPR1<br>BraraA01_NPR1     | 140 150<br>VSFDSVVIVLAYVYSSRVRPPPKGV<br>G. A   | 160 170<br>SECADDNOR BACRPAVDER 1<br>  | 180 190<br>EVLYLAFTEKO ELVKLYORH<br>N T.<br>N T.<br>  | 200         210         220           LLR TVDR         V5         100         100           DV         V         1         100         100           DV         V         1         100         100         100           DV         V         1         100         100         100         100           DV         V         1         100         100         100         100         100           DV         V         1         100 <t< td=""><td>230         240         250         260           RL DRC IE I IV SD VD KVSL KSLPB IVR0II         E        </td></t<> | 230         240         250         260           RL DRC IE I IV SD VD KVSL KSLPB IVR0II         E   |
| Cr1504_NPR1<br>Cg926_NPR1<br>Co_NPR1<br>Np_NPR1<br>Aly_NPR1<br>At_NPR1<br>BraraA03_Npr1<br>BraraA08_MPR1<br>BraraA06_NPR1<br>BraraA01_NPR1 | 270         280           GKRKELGSEVPELEIMUSRIHKALD         N.           N.         S.           J.         T. K.           N.         S.           J.         K. NL           DN.         S.           J.         J.K.           DN.         S.           J.         J.K.           DR.         J.K.           J.K.         N.           J.K.         K.           J.K.         K.           J.K.         N.           J.         J.K.           J.         S.           J.         S.           J.         S.           J.         J.K.           J.         J.K.           J.         J. N.  | 290 300<br>SDDTALVSLLITEGHTNLDDA<br>VA. EM. S. V<br>VZ. KI. K. S. V<br>VZ. K. K. S. S. V<br>VZ. K. K. S.   | 310 320<br>SALHFAVAYCDVKYAJDLI KI<br>   | 330 340 350<br>D ADVN RN SRUTULHVAMRKEPOLT<br>. N. H. S.<br>KI. H. S.<br>KI. H. S.<br>KI. J. J. S.<br>S. S. S. S.<br>S. S. S   | 360         370         380         390           LSLUPKCARASETVILEGRTALLIARENTAAUSYNN         T         55         M         C         N           T         SE         M         C         N         C         N         N           T         SE         M         C         N         C         N         N           T         SE         M         C         N         C         N         N           N         D         S         M         C         N         C         N           A         N         D         S         N         C         C         N         C           A         T         N         D         S         QV         A         A           A         T         N         D         QV         A         A           A         T         N         D         QV         A         A           A         T         N         D         QV         A         A |
| Cr1504_NPR1<br>Cg926_NPR1<br>Co_NPR1<br>Np_NPR1<br>Aly_NPR1<br>At_NPR1<br>Thhal_NPR1<br>BraraA03_Npr1<br>BraraA06_NPR1<br>BraraA01_NPR1    | 400 410<br>VE2OSRISCRGRVCVCILEE-ADRA<br>C.I.L.L.EC.C.<br>RYI.L.L.EC.C.<br>RYI.L.L.EC.C.<br>RYI.L.L.EC.C.<br>RYI.L.L.EC.C.<br>RYI.L.L.EC.C.<br>RYI.L.L.EC.C.<br>IIC.I.L.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.L.EC.C.<br>IIC.I.I.E   | 420 430<br>VTIPTDAPEVAMAK-ELK<br>EP. R.V. S V. D<br>EP. R.V. S V. D<br>EP. R.V. S V. D<br>20. R.V. S-FTV. D<br>20. R.V. S-FTV. D<br>20. R.V. S-TV. D<br>EP. R.V. S.S<br>DFF R.VS.S<br>DFF. P. VF.S<br>DF. P. D. S.S.S. | 440 450<br><b>PRLIDLENRVATAORLIFEVEN</b><br><b>F. I</b><br><b>F. I</b><br><b>I</b><br><b>F. I</b><br><b>I</b><br><b>I</b><br><b>I</b><br><b>I</b><br><b>I</b><br><b>I</b><br><b>I</b> | 460         470         480           CMAMETA OHKGTOEFIVTSDEPDHUTGK         G.           V.         S.           V.         S.           V.         V.           V.         S.           V.         S.           V.         S.           V.         S.           V.         S.           V.         D.           V.         S.           V.         D.           V.         D.  | 490         500         510         522           KRTSEDVDLAPEKIDEEHCSRLKALTKTVPICKRF         S.         S.         S.   |
| Cr1504_NPR1<br>Cr3926_NPR1<br>Co_NPR1<br>Np_NPR1<br>Al_y_NPR1<br>At_NPR1<br>Thhal_NPR1<br>BraraA03_Npr1<br>BraraA03_NPr1<br>BraraA01_NPR1  | 530 540  FPRCSPVLDR IMPCODIATIACCE A.V.OTIIDE.ONM. A.V.OTIIDE.O.M. A.OTIECOMMAN. A.OTIECOMM. A.OTIECOM | 550 560  | 570 580<br>KRAFAECKL-IRNSSHAASSS<br>NNIGRT<br>NSIGRT<br>NSIF<br>NSIF<br>NSN.FGK.IT.A.<br>S.N.GLTD.T.<br>E.FG.LS<br><br>E.HG.LS<br>M.N.NE.RGK.RSC<br>C.S.K.EDLGK.LS  | 590 600 610   |  |

Supplementary Figure 4: NPR1 phylogeny in Brassicaceae

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**a**, **b** Phylogenetic tree (**a**) and protein sequence alignment (**b**) of NPR1 orthologues from different Brassicaceae. Identical amino acids are indicated by dots; conservative changes by grey, non-conservative changes by black shading. Aly: *Arabidopsis lyrata*, Ath: *A. thaliana*, Brara: *Brassica rapa*, Np: *Neslia paniculata*, Thhal: *Thelungiella halophila*.





# Supplementary Figure 5: Haplotype structure of *NPR1* and *RPP5* in *C. grandiflora* and *C. rubella*.

**a** Haplotype network of *NPR1* in *C. grandiflora* (green), *C. rubella* (yellow), *C. orientalis* (blue) and *N. paniculata* (red). Red outlines indicate the *NPR1* haplotypes segregating in the RIL population. Analysis is based on resequencing of a 700 bp fragment.

**b** Neighbor-joinng phylogenetic tree of *RPP5* haplotypes from different *C. grandiflora* and *C. rubella* accessions (left) and corresponding haplotype network (right). Analysis is based on resequencing of an 800 bp fragment. Numbers indicated bootstrap support from 1,000 runs.

**c** Genome structure of *RPP5* region in Brassicaceae. Colours indicate syntenic genes. The phylogenetic tree shows known evolutionary relationships between the species. Corresponding regions from the three *B. rapa* subgenomes are shown.



### Supplementary Figure 6: Extended NPR1 and RPP5 phylogenies.

Phylogenetic trees for *NPR1* (top) and *RPP5* (bottom) including additional accessions used for crosses in Supplementary Figure 7. Incompatible haplotypes are indicated in red, compatible ones in blue and haplotypes not tested in grey. Analyses are based on resequencing of 700 bp (*NPR1*) and 800 bp fragments (*RPP5*). Numbers indicated bootstrap support from 1,000 runs. Red font indicates the alleles that have been tested in crossing experiments and shown to be incompatibles; green font indicates alleles that have been tested in crossing experiments and shown to be compatible; and black font indicates the alleles that have not been tested.

| 0            | C. grandiflora | C. rubella  | Ed annual mat | % incompatible | 4-4-1 | n                     |                         |
|--------------|----------------|-------------|---------------|----------------|-------|-----------------------|-------------------------|
| Cross number | accession      | accession   | F1 genotype"  | pnenotype      | total | strongly incompatible | moderately incompatible |
| 1            | Cg 2           | Cr 1504/2   | 2gr;7gr       | 9.24           | 119   | 1                     | 10                      |
| 5            | Cg 93          | Cr 1504/2   | 2gr;7gr       | 22.12          | 113   | 7                     | 18                      |
| 6            | Cg 89          | Cr 1504/2   | 2gr;7gr       | 32.32          | 99    | 9                     | 23                      |
| 7            | Cg 88          | Cr 1504/2   | 2gr;7gr       | 10.26          | 117   | 0                     | 12                      |
| 11           | Cg 88/2        | Cr 1504/2   | 2gr;7gr       | 20.00          | 95    | 0                     | 19                      |
| 16           | Cg 5           | Cr 1504     | 2gr;7rr       | 8.30           | 253   | 0                     | 21                      |
| 23           | Cg 93/2        | Cr 1504     | 2gr;7gr       | 12.35          | 251   | 14                    | 17                      |
| 26           | Cg 926         | Cr 1504     | 2gr;7gr       | 25.00          | 148   | 8                     | 29                      |
| 30           | Cg 926/2       | Cr 1504     | 2gr;7gr       | 18.60          | 129   | 4                     | 20                      |
| 2            | Cg 88          | Cr 4.23     | 2gr;7gr       | 0              | 110   | 0                     | 0                       |
| 9            | Cg 88/3        | Cr 4.23     | 2gr;7gr       | 20.25          | 79    | 8                     | 8                       |
| 4            | Cg 926/3       | Cr 86/T1-C  | 2rr;7rg       | 0              | 97    | 0                     | 0                       |
| 12           | Cg 926/4       | Cr 22.5     | 2m; 7rg       | 0              | 119   | 0                     | 0                       |
| 21           | Cg 88/2        | Cr 22.5     | 2gr;7gr       | 3.88           | 103   | 3                     | 1                       |
| 3            | Cg 94          | Cr 1GR1-TS1 | 2gr;7gg       | 22.03          | 177   | 20                    | 19                      |
| 20           | Cg 83          | Cr 1GR1-TS1 | 2gr;7gg       | 0              | 132   | 0                     | 0                       |
| 24           | Cg 88          | Cr 1GR1-TS1 | 2gr;7gg       | 0              | 129   | 0                     | 0                       |
| 8            | Cg 89          | Cr 1377/5   | 2gr;7gr       | 0              | 126   | 0                     | 0                       |
| 10           | Cg 5           | Cr 1377/5   | 2gr;7gr       | 17.75          | 169   | 14                    | 16                      |
| 13           | Cg 2h          | Cr 1377/5   | 2gr;7gr       | 0              | 129   | 0                     | 0                       |
| 25           | Cg 94          | Cr 1377/5   | 2gr;7gr       | 0              | 135   | 0                     | 0                       |
| 27           | Ca 5/2         | Cr 1377/5   | 2ar;7ar       | 0              | 138   | 0                     | 0                       |

b

Extreme Phenotype

\* for QTL2, "r" denotes an NPR1rub allele, "g" denotes an NPR1go allele; for QTL7, "r" denotes the presence of RPP5, "g" denotes its absence Phenotype Genotype Extreme Phenotype Phenotype

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| Extreme Phenotype | Phenotype   | Genot | ype   |  | Ext     | reme Phenotype      | Phenotype  | Geno | type  |  |
|-------------------|---|-------|-------|--|---------|---------------------|--|------|-------|--|
| Inc. Comp.        | nequency  | Inc.  | Comp. |  | Inc.    | Comp.               | inequency  | Inc. | Comp. |  |
| Cross1            | 100% •<br>100% •<br>100% •<br>50% •<br>10% •<br>10% •<br>10% •                  |       |       | 1×10 <sup>-4</sup><br>3×10 <sup>-4</sup>   | Cross23 |                     | 300% 4<br>80% 4<br>70% 4<br>50% 4<br>50% 4<br>40% 5<br>30% 4<br>10% 4<br>0% 4          |      |       | 2x10 <sup>-21</sup><br>1x10 <sup>-89</sup> |
| Cross5            | 100%<br>30% -<br>80% -<br>70% -<br>60% -<br>55% -<br>40% -<br>10% -<br>0% -     |       |       | 5x10 <sup>-22</sup>                        | Cross11 | K                   | 100% 9<br>30% 4<br>80% 4<br>70% 4<br>60% 4<br>30% 4<br>30% 4<br>30% 4<br>20% 4<br>0% 9 |      |       | 7x10 <sup>20</sup><br>4x10 <sup>-9</sup>   |
| Cross6            | 100% *<br>100% *<br>80% *<br>50% *<br>40% *<br>10% *<br>10% *                   |       |       | 6x10 <sup>-61</sup><br>2x10 <sup>-3</sup>  | Cross16 |                     | 200% 4<br>80% 4<br>80% 4<br>70% 4<br>60% 4<br>30% 4<br>90% 4<br>20% 4<br>0% 4          |      |       | 8x10 <sup>-64</sup><br>2x10 <sup>-4</sup>  |
| Cross7            | 100% =<br>90% =<br>80% =<br>70% =<br>50% =<br>40% =<br>10% =<br>10% =<br>00% =  |       |       | 3x10 <sup>-4</sup><br>3x10 <sup>-4</sup>   | Cross3  | X                   | 100% 0<br>50% 4<br>60% 4<br>60% 4<br>60% 4<br>60% 4<br>60% 4<br>20% 4<br>10% 4<br>0% 4 |      |       | 8x10 <sup>-12</sup><br>9x10 <sup>-08</sup> |
| Cross26           | 100%, *<br>30% -<br>80% -<br>50% -<br>50% -<br>30% -<br>30% -<br>10% -<br>0%, * |       |       | 3x10 <sup>-10</sup><br>5x10 <sup>-46</sup> | Cross9  | A.                  | 200% 4<br>80% 4<br>80% 4<br>60% 4<br>60% 4<br>30% 4<br>30% 4<br>20% 4<br>0% 4          |      |       | 0  |
| Cross30           | 100% *<br>90% *<br>80% *<br>60% *<br>50% *<br>10% *<br>10% *                    |       |       | 1×10 <sup>-21</sup><br>3×10 <sup>-10</sup> | Cross10 | X                   | 300% 4<br>80% 4<br>80% 4<br>80% 4<br>50% 4<br>30% 4<br>30% 4<br>30% 4<br>30% 4         |      | PR1   | 3x10 <sup>-15</sup><br>0.27                |
| Wild              | type  | 2gg   | 2rg   | 2rr  |         | Wild t              | ype  | 2gg  | 2rg   | 2rr  |
| Moderate incompat | ibility   | RI    | PP5   |  |         | Moderate incompatit | pility   | RF   | PP5   |  |
| Strong incompat   | ibility   | 7gg   | 7rg   | 7rr  |         | Strong incompatit   | pility   | 7gg  | 7rg   | 7rr  |

# Supplementary Figure 7: Incompatible phenotypes in additional *C. rubella* x *C. grandiflora* crosses.

**a** Table of additional crosses performed and proportion of incompatible phenotypes in resulting F2 populations. Blue font indicates crosses in which no *RPP5* was segregating. Note that the absence of incompatible phenotypes in crosses 4 and 12 is not informative, as the *C. grandiflora* parental plants of these crosses did not pass on an *NPR1<sup>go</sup>* allele to the respective F1 plants. This is due to the *C. grandiflora* accession *Cg926* being heterozygous for *NPR1<sup>go</sup>/NPR1<sup>rub</sup>* (see Fig. 3a).

**b** Phenotypes and genotypes of compatible and incompatible plants in F2 populations from crosses in (a). Numbers indicate the p-values determined by Chi-square test comparing genotype frequencies in the compatible and incompatible cohort. The sample size for the calculation of the phenotype frequency is indicated in figure 7a, n = 16 - 38 and n = 8 - 44 for the genotype frequency of the compatible and incompatible phenotypic classes respectively.



Supplementary Figure 8: Characterization of an additional *NPR1*-linked, but *RPP5*independent incompatibility between *C. grandiflora* and *C. rubella*, and of the incompatibility between *C. rubella* and *C. orientalis*.

**a** Expression of the indicated immune-response markers determined by qRT-PCR normalized to *Capsella TUB6* in compatible and incompatible plants from cross 3 in Supplementary Figure 7. Mean

 $\pm$  s.e.m. of three biological replicates is shown. Asterisks indicate significant differences at \* p<0.05 and \*\* p<0.01 based on a two tailed Student's t-test.

**b** Growth retardation in incompatible genotypes from cross 3 in Supplementary Figure 7 is alleviated by higher ambient temperatures.

**c** *C. orientalis Co1983* x *C. rubella Cr1504* (*Co* x *Cr*) F1 hybrids show an incompatible phenotype, whereas *C. orientalis Co1983* x *C. grandiflora Cg926* (*Co* x *Cg*) F1 hybrids do not.

**d** Phenotype distribution in different genotype classes of F2 plants from the *C. rubella 1504* x *C. orientalis* cross (n=144). For the *NPR1* genotype on chromosome 2, 'r' refers to the *NPR1<sup>rub</sup>* allele and 'g' refers to the *NPR1<sup>go</sup>* allele; for the *RPP5* genotype on chromosome 7, 'r' refers to the presence of *RPP5*, while 'g' refers to the deletion allele.

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| Bin       | Name  | Elements | Odds ratio | P-value   |
|-----------|---|----------|------------|-----------|
| 30.2.11   | signalling.receptor kinases.leucine rich repeat XI  | 209      | 3.623193   | 0.0001604 |
| 29.4.1.57 | protein.postranslational modification.kinase.receptor like cytoplasmatic kinase VII                   | 318      | 2.6974307  | 0.0018602 |
| 29.4.1    | protein.postranslational modification.kinase  | 326      | 2.62425436 | 0.0019645 |
| 20.1      | stress.biotic   | 615      | 2.05602373 | 0.0038048 |
| 20        | stress  | 1100     | 1.68163609 | 0.0154877 |
| 26.8      | misc.nitrilases, *nitrile lyases, berberine bridge enzymes, reticuline oxidases, troponine reductases | 102      | 3.69128104 | 0.0167027 |
| 29.4      | protein.postranslational modification   | 1027     | 1.61747364 | 0.0488519 |
|           |   |          |            |           |



**MASTA** experiment

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#### Supplementary Figure 9: Transcriptome effects of different NPR1 allele clades.

**a** Table of overrepresented (p<0.05) MapMan categories amongst the 1000 genes with the lowest p-values when testing for association between gene expression and *NPR1* genotype. Categories were identified using a Fisher test. Bin and Name describe the categories, elements gives the number of genes of the category. P-values are BH corrected.

**b** Distributions of gene-overlap counts between the 1000 genes with the lowest p-values when testing for association between gene expression and *NPR1* genotype and *Arabiodpsis thaliana* microarray experiments grouped according to experiment type (n= 2-167). Overlaps were determined using MASTA; experiment type is based on MASTA classification. Asterisks mark significant difference from overlap counts with all experiments at p<0.05 based on Wilcoxon rank-sum test. n indicate the number of experiments per category. Pathogen experiments were the most strongly significant ones (p<1e-11) Dashed grey line represents median overlap for all experiments.

### Supplementary Table 1: Summary results of the QTL mapping for stunted growth in the Cg926

x Cr1504 RILs.

| QTL  | location in cM<br>(closest marker) | LOD    | 2LOD Confidece interval in cM (surronding markers) | Additive<br>effect | Phenotypic<br>variance<br>explained (%) |
|------|------------------------------------|--------|--|--------------------|---|
| QTL2 | 0                                  | 20.522 | 0-5 (cap5-1g63)                                    | -0.36              | 41.261                                  |
| QTL7 | 35                                 | 11.694 | 30-45 (g06-g08)                                    | 0.26               | 15.462                                  |

The additive effect was calculated as half of the difference of the phenotypic averages between Cr and Cg homozygote genotypes derived from the coding scheme +1 for Cr and -1 for Cg.

| Scaffold | Position (nt) | Marker (see ref. 1) | Genotype of NIL SAS437<br>(a: homozygous <i>C. grandiflora</i> ;<br>b: homozygous <i>C. rubella</i> ;<br>h: heterozygous) |
|----------|---------------|---------------------|---|
| 1        | 2299856       | A05                 | b   |
| 1        | 3114855       | A07                 | b   |
| 1        | 4555967       | A08                 | b   |
| 1        | 6168749       | A10                 | b   |
| 1        | 8858360       | A12                 | h   |
| 1        | 9766939       | 3G33                | b   |
| 1        | 19167208      | A16                 | b   |
| 2        | 363608        | CAP5                | h   |
| 2        | 403722        | B01                 | h   |
| 2        | 763403        | 1G63                | b   |
| 2        | 955458        | B02                 | b   |
| 2        | 1587177       | 1G61                | b   |
| 2        | 7388511       | B04                 | b   |
| 2        | 7816098       | B06                 | b   |
| 2        | 9064596       | B07                 | b   |
| 2        | 9604872       | B08                 | b   |
| 2        | 12200809      | B11                 | b   |
| 2        | 13270845      | B10                 | b   |
| 2        | 201420        | 2002                | 1   |
| 3        | 381439        | 3G02                | b   |
| 3        | 6151596       | 3G17                | b   |
| 3        | 7559574       | C004                | b   |
| 3        | 13241733      | C06                 | b   |
| 3        | 13513239      | C10                 | b   |
| 4        | 1741932       | D03                 | h   |
| т<br>Д   | 700/190       | D03                 | b   |
|          | 728/563       | D04                 | h   |
| 4        | 8622170       | 2032                | h   |
| 4        | 8032170       | 2032                | υ   |

## Supplementary Table 2: Genotype of the near-isogenic line

| 4 | 13451362 | D08    | b |
|---|----------|--------|---|
|   |          |        |   |
| 5 | 34889    | C07    | h |
| 5 | 1824372  | E02    | h |
| 5 | 5274426  | E05    | h |
| 5 | 7709629  | E04    | h |
| 5 | 9974165  | E08    | h |
| 5 | 13584056 | CAP175 | b |
|   |          |        |   |
| 6 | 5967395  | F03    | h |
| 6 | 9368196  | F6     | a |
| 6 | 15009532 | 4G05   | b |
| 6 | 16513470 | F12    | b |
| 6 | 16525594 | F11    | b |
|   |          |        |   |
| 7 | 2298776  | G02    | b |
| 7 | 4512247  | G03    | h |
| 7 | 9972054  | 4G15   | h |
| 7 | 9569806  | G07    | h |
| 7 | 10149356 | G08    | h |
| 7 | 15915126 | G12    | b |
| 7 | 16968217 | G11    | h |
|   |          |        |   |
| 8 | 1078892  | H03    | b |
| 8 | 2623520  | H04    | b |
| 8 | 11130390 | H10    | b |
| 8 | 13265528 | H11    | b |

| QTL | Scaffold   | Start   | Stop    | ID              | A. thaliana homologues                   | Functional annotation   |
|-----|------------|---------|---------|-----------------|--|---|
|     | scaffold_2 | 202551  | 204786  | Carubv10021457m | AT1G64300.1                              | Protein kinase family protein   |
|     |            |         |         |                 |  | F-box associated ubiquitination effector family   |
|     | scaffold_2 | 211957  | 213051  | Carubv10021816m | AT1G64295.1                              | protein   |
|     | scaffold_2 | 215677  | 216762  | Carubv10021907m | AT1G64290.1                              | F-box protein-related   |
|     | scaffold_2 | 217298  | 219989  | Carubv10020031m | AT1G64280.1_NPR1                         | regulatory protein (NPR1)   |
|     | scaffold_2 | 220545  | 222704  | Carubv10020278m | AT1G64260.1                              | MuDR family transposase   |
| 2   | scaffold_2 | 223775  | 225895  | Carubv10021357m | AT1G64260.1                              | MuDR family transposase   |
| II  | scaffold_2 | 227573  | 229999  | Carubv10021783m | AT1G64260.1                              | MuDR family transposase   |
| 0   | scaffold_2 | 230895  | 233624  | Carubv10020053m | AT1G64255.1                              | MuDR family transposase   |
|     | scaffold_2 | 234743  | 235063  | Carubv10021722m | AT1G64235.1                              | Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein |
|     | scaffold_2 | 235451  | 237355  | Carubv10020996m | AT1G64230.1_UBC28                        | ubiquitin-conjugating enzyme 28   |
|     | scaffold_2 | 237595  | 238024  | Carubv10021245m | AT1G64220.1_TOM7-2                       | translocase of outer membrane 7 kDa subunit 2   |
|     | scaffold_2 | 238102  | 240015  | Carubv10021395m | AT1G64210.1                              | Leucine-rich repeat protein kinase family protein   |
|     | scaffold_2 | 241053  | 242892  | Carubv10020883m | AT1G64200.1_VHA-E3                       | vacuolar H+-ATPase subunit E isoform 3  |
|     | scaffold_7 | 9253104 | 9254894 | Carubv10005176m | AT4G08691.1                              |   |
|     | scaffold_7 | 9254981 | 9258150 | Carubv10005361m | AT4G17050.1_UGLYAH                       | ureidoglycine aminohydrolase  |
|     | scaffold_7 | 9257045 | 9262244 | Carubv10005166m | AT4G17040.1_CLPR4                        | CLP protease R subunit 4  |
|     | scaffold_7 | 9264180 | 9265793 | Carubv10005585m | AT4G17030.1_AT-EXPR                      | expansin-like B1  |
|     | scaffold_7 | 9266354 | 9269322 | Carubv10006310m | AT4G17020.3                              | transcription factor-related  |
| IL  | scaffold_7 | 9268573 | 9271765 | Carubv10005920m | AT4G17010.1                              |   |
| ð   | scaffold_7 | 9272660 | 9275533 | Carubv10006518m | AT4G17000.1                              |   |
|     | scaffold_7 | 9277237 | 9277809 | Carubv10006767m | AT4G16980.1                              | arabinogalactan-protein family  |
|     | scaffold_7 | 9279776 | 9284122 | Carubv10004105m | AT4G16970.1                              | Protein kinase superfamily protein  |
|     | scaffold_7 | 9291754 | 9296295 | Carubv10004008m | AT4G16890.1_BAL,SNC1<br>AT4G16950.1_RPP5 | disease resistance protein (TIR-NBS-LRR class), putative                                  |
|     | scaffold_7 | 9297395 | 9299037 | Carubv10005274m | AT4G16850.1                              |   |

Supplementary Table 3 : List of annotated genes in the mapping intervals.

| scaffold_7 | 9299185 | 9303240 | Carubv10004893m | AT4G16845.1_VRN2        | VEFS-Box of polycomb protein                    |
|------------|---------|---------|-----------------|-------------------------|---|
| scaffold_7 | 9303846 | 9304112 | Carubv10007592m | AT4G16840.1             |   |
|            |         |         |                 |                         | Tetratricopeptide repeat (TPR)-like superfamily |
| scaffold_7 | 9304231 | 9306290 | Carubv10006791m | AT4G16835.1             | protein   |
| scaffold_7 | 9306420 | 9308554 | Carubv10005134m | AT4G16830.1             | Hyaluronan / mRNA binding family                |
| scaffold_7 | 9309482 | 9311038 | Carubv10007507m | AT4G16820.1_PLA-Ibeta2  | alpha/beta-Hydrolases superfamily protein       |
| scaffold_7 | 9316581 | 9318771 | Carubv10006259m | AT4G16810.1             | VEFS-Box of polycomb protein                    |
| scaffold_7 | 9321931 | 9324324 | Carubv10007038m | AT4G16810.1             | VEFS-Box of polycomb protein                    |
| scaffold_7 | 9325020 | 9327589 | Carubv10004885m | AT4G16807.1             |   |
|            |         |         |                 |                         | ATP-dependent caseinolytic (Clp)                |
| scaffold_7 | 9328471 | 9329520 | Carubv10007120m | AT4G16800.1             | protease/crotonase family protein               |
| scaffold_7 | 9332345 | 9334066 | Carubv10004732m | AT4G16790.1             | hydroxyproline-rich glycoprotein family protein |
| scaffold_7 | 9335010 | 9336719 | Carubv10005275m | AT4G16780.1_ATHB-2      | homeobox protein 2                              |
|            |         |         |                 |                         | 2-oxoglutarate (2OG) and Fe(II)-dependent       |
| scaffold_7 | 9345932 | 9348909 | Carubv10005289m | AT4G16770.1             | oxygenase superfamily protein                   |
|            |         |         |                 |                         | 2-oxoglutarate (2OG) and Fe(II)-dependent       |
| scaffold_7 | 9349617 | 9353002 | Carubv10007744m | AT4G16770.1             | oxygenase superfamily protein                   |
|            |         |         |                 |                         | 2-oxoglutarate (2OG) and Fe(II)-dependent       |
| scaffold_7 | 9353682 | 9356075 | Carubv10006983m | AT4G16765.1             | oxygenase superfamily protein                   |
| scaffold_7 | 9357182 | 9361483 | Carubv10004312m | AT4G16760.1_ACX1,ATACX1 | acyl-CoA oxidase 1                              |
| scaffold_7 | 9364548 | 9365366 | Carubv10005867m | AT4G16750.1             | Integrase-type DNA-binding superfamily protein  |
| scaffold_7 | 9374054 | 9375936 | Carubv10007826m | AT4G16745.1             | Exostosin family protein                        |

| C. grandiflora population | City, country    | Latitude/Longitude |  |
|---------------------------|------------------|--------------------|--|
| - <b>-</b>                | Kastanonas,      |                    |  |
| P19                       | Greece           | 39,84N/20.95E      |  |
| Cg 926/2                  | Votonosi, Greece | 39.76N/21.11E      |  |
| P18                       | Vovusa, Greece   | 39.91N/21.05E      |  |
| P11                       | Metsovo, Greece  | 39,77N/21,18E      |  |
| P14                       | Metsovo, Greece  | 39.76N/21.18E      |  |
| P22                       | Konitsa, Greece  | 40.05N/28.89E      |  |
|                           |                  |                    |  |

Supplementary Table 4: Geographic origin of the additional *C. grandiflora* populations collected during this study.

### Supplementary Table 5: List of the primers used in the study

|         |                             | Position;             |  |
|---------|-----------------------------|-----------------------|--|
| Nomo    | Usaga                       | Polymorphism<br>Cr/Ca | Sequence (5' 3')   |
| Ivallie | Cloning of NPR1             | Circg                 | TTAATTAA   |
| oAS1036 | ORFs                        | n.a.                  | ATGGACACCAATCTTGATGGATTCGC   |
| oAS1037 | Cloning of NPR1<br>ORFs     | n.a.                  | TTAATTAATCACCGACGCCGATTAGAGAGTT<br>T   |
| oAS1137 | Cloning of the<br>173tsRPP5 | n.a.                  | CTCCTCTTAATTAAGTGATTTTTCTCTACAAG<br>CGAATCTAGAGAGGATATTCGAGAAGTATTG<br>ACCGATA |
| oAS1138 | Cloning of the<br>173tsRPP5 | n.a.                  | CTCCTCTTAATTAATTGAGACTTCCAAGTCGC<br>AGAGTTCC                                   |
| oAS1129 | Genotyping of NPR1          | n.a.                  | TTGATGCTCTTCTAGGATTTTGAAAGGTGCTA   |
| oAS1130 | Genotyping of NPR1          | n.a.                  | GACTCGGATGATATCACGCTAGTCAATTTGC  |
| oAS873  | Genotyping CrRPP5           | n.a.                  | CAAACCATAATAGTAAGATGCTTCAGTTACC<br>AG  |
| oAS1123 | Genotyping CrRPP5           | n.a.                  | TCGAATACCTCGATCTCAGTGGTT   |
| oAS839  | genotyping CgRPP5           | n.a.                  | GCTGTTGATGCTTAAGGAGGTTCCC  |
| oAS1133 | genotyping CgRPP5           | n.a.                  | GTTAATGATGGGAAGAACACCTTTTAC  |
| oAS1276 | genotyping CgRPP5           | n.a.                  | ATGAAAATCTCCACTCAAGTAGTGTCCACAC  |
| oAS1072 | qPCR CaNPR1                 | n.a.                  | CACCGAGTCCTCTATCGTTTATC  |
| oAS1073 | qPCR CaNPR1                 | n.a.                  | GTCAGCGAGAACGAGCTTAG   |
| oAS1084 | qPCR caTUB6                 | n.a.                  | GCTCCTTCAGTGTAGTGCCC   |
| oAS1085 | qPCR <i>caTUB6</i>          | n.a.                  | CAGAACTGGCCCTTATGGTC   |
| oAS1068 | qPCR CaRPP5                 | n.a.                  | GACGATGAGATTAAAATGACTTCTGCCA   |
| oAS1069 | qPCR CaRPP5                 | n.a.                  | AGCGCCTTGAGAAGATGACTAAGGAA   |
| oAS1145 | qPCR<br>Carubv10025432m     | n.a.                  | GTAACCTGTACCAATTTGTACCCATC   |
| oAS1146 | qPCR<br>Carubv10025432m     | n.a.                  | TGTAGAGATGCCATCCTGGCG  |
| oAS1074 | qPCR CaPR1                  | n.a.                  | TTGCAACTGATGATGGTTCC   |
| oAS1075 | qPCR CaPR1                  | n.a.                  | TAGTGGCGACTTGTCTGGTG   |
| oAS1111 | qPCR CaEDS5                 | n.a.                  | TCTTTATTGGTTTGGTTTGTGG   |
| oAS1112 | qPCR CaEDS5                 | n.a.                  | CAGTAACAGCCCAAGGTCC  |
| oAS1113 | qPCR CaEDS1                 | n.a.                  | TCGGAAGGAGAAATACACGG   |
| oAS1114 | qPCR CaEDS1                 | n.a.                  | GCTCTTACCGGAATCAATGG   |
| oAS1078 | qPCR CaPDF1.2               | n.a.                  | TTATGCGAGAGGTCAAGTGG   |
| oAS1079 | qPCR CaPDF1.2               | n.a.                  | GATCCATGTCGTGCTCCTTC   |
| oAS1107 | qPCR PR2                    | n.a.                  | TCTCAGACACCCCGATCTTC   |
| oAS1108 | qPCR PR2                    | n.a.                  | CCACATGTATAACTCGGGCC   |
| oAS1109 | qPCR CaPAD4                 | n.a.                  | AAGAAAGGCAGCACATAGATCC   |
| oAS1110 | gPCR CaPAD4                 | n.a.                  | ACAAAGCTCGCGAAGAGAAG   |
| oAS1080 | gPCR CaORA59                | n.a.                  | ACCGCCCTAGAGGAAAGAAG   |
| oAS1081 | gPCR CaORA59                | n.a.                  | TCACTTTCTTGCGTCGTGAC   |
|         | HiB1 CAPS markers           |                       | GGTTCGTCGGCGCACACATTGAG  |
| oAS928  | using <i>HindII</i>         | scaffold_2:75028;     |  |
| oAS929  | using <i>HindII</i>         | C/I                   |  |

| oAS926     markers using Clal     scaffold 2:129775;     TCGAACGGCAATCTTCAAGTTCATCGA       HB3 CAPS markers     C/T     CTATTCTCGACGACGAGCACGAGGTTGTC       HB3 CAPS markers     A/G     AGTCAGAAGCAGGAGCACGAGAGTTGTC       oAS1000     using Mall     CACACCTGATCCATAAATCTGGTATGCAC       HB4 CAPS markers     a/G     AGCCAGAGCAGAGAGCAGAGAGAGAGAGAAGAATT       oAS1030     using Ddel     AACACTGAAGACAAGAAGAAGAAGAATT       oAS1040     using Ddel     AACAATCAAGATCAAGAAGAAGAAGAAGAATT       HB5 CAPS markers     G/A     AAGATCAAGATCAAGAGAGAGAGAGAAGAATT       HB5 CAPS markers     G/T     GTTCTGGAATTTCCCACTGGTCGT       oAS901     using Abd     AAGATCAAGATCAAGAAGAGAGAGAGAGAGGG       AS914     GTTCTGGAATTTGCAATGAGAGTGAGGG       HB5 CAPS markers     G/C     GTTGTGGACTCTAGCATAGAAGAGTGATGGG       AS914     GTTCTGGAATTTCCCAATGGACATGGAAGAGGGGGGGAAGAAGAATT       HB6 CAPS markers     G/C     GTGTGACCTTAGCATAGAAGAGTGAGGGGGGAAGAAGACTGA       HB7 dCAPS     G/A     GTGACCTTAGCATAGAACAGAGCACAGCC       HB8 dCAPS     G/A     CTACCTCTTGGTCACACTCA       GAS910     markers using Ddel     scaffold 2:30508 ;     TCACCTCTTGGTACACTCAGAACAGCCACTCA       HB8 dCAPS     G/A     CTACCTCTTGGTCACACTGCA       CAPS F     markers using Ddel     scaffold 7:913785 ;     ACACCTGGTGCACTTAGCACAGCACAGC   |            | HiB2 dCAPS                |                      |                                 |
|---|------------|---------------------------|----------------------|---------------------------------|
| HB2 GAPS         CT           0AS927         markers using Clal         CTATTCTCGACGACGAGCTTCTAAG           0AS1001         using Mall         scaffold_2.203131;         AGTCAGAAGCAGGACCAGAGAGTGTC           0AS1002         using Mall         Scaffold_2.210313;         AGTCAGAAGCAGGACCAGAGAGTGTC           0AS1002         using Mall         Scaffold_2.211249;         CACACGATCCACAGAAGAGGAGAGAGAAGAATT           HB3 CAPS markers         G/A         AAGTCAAGAATCAAGAGAGAGAGAGAGAAGAATT           HB3 CAPS markers         Scaffold_2.2249356;         CGGTTCAGTACTTTAAGAAGAGGAGAGAGAGAGAGAGAGA   | oAS926     | markers using ClaI        | scaffold_2:129775;   | TCGAACGGCAATCTTCAAGTTCATCGA     |
| oAS927       markers using Clai       CTATTCTCGACGACGAGCTTCTAAG         HB3 CAPS markers       scaffold _2:203131;       AGTCAGAAGCAGGACCAGAGAGTTGTC         AIB       AIG       CACACCTGATCCTATAATCTGGTATGCAC         HB4 CAPS markers       scaffold _2:241249;       AACCAGATCCACAGAGAGAGAGAGAGAAGTT         HB4 CAPS markers       G/A       AAGATCAAGAATCAAGAGAGAGAGAGAGAAGAATT         HB5 CAPS markers       G/A       AAGATCAAGAATCAAGAGAGAGAGAGAGAGAAGAATT         HB5 CAPS markers       CT       GTCTGGAATTCCCAATAGAGAGAGAGAGAGAGAGAGAGAG  |            | HiB2 dCAPS                | C/T                  |                                 |
| HiB3 CAPS markers<br>oAS1001       scaffold 2:203131 ;<br>HiB3 CAPS markers<br>oAS102       scaffold 2:203131 ;<br>A/G       AGTCAGAAGCAGGACCAGAGAGATGTCC         0AS1002       using Nattr<br>WiB3 CAPS markers<br>oAS1039       scaffold 2:241249 ;<br>G/A       AACCAGATCCACCAGATGGTCGCT         0AS1040       using Datel       scaffold 2:241249 ;<br>G/A       AACCAGATCCACCAGAGAGAGAGAGAGAATT         HiB5 CAPS markers<br>oAS1040       using Datel       scaffold 2:249356 ;<br>C/T       CGGTTCAGTACTTAAGAAGAGAGAGAGAGAGATT         HiB5 CAPS markers<br>oAS911       scaffold 2:249356 ;<br>Using Apol       CGGTTCAGAATTGCCAATGGAGAGAGAGAGGGGGGG         HiB6 CAPS markers<br>oAS912       scaffold 2:252505 ;<br>HiB6 CAPS markers       TAGTGACATTGACATAGAAGAGGAGAGAGGGGGGGGGGG   | oAS927     | markers using ClaI        |                      | CTATTCTCGACGACGAGCTTCTAAG       |
| oAS1001       using Natlit       scaffold_2:203131;       AGTCAGAAGCAGGACCAGAGAGTGTCC         oAS1002       using Natlit       CACACCTGATCCTATAATCTGGTATGCAC         oAS103       using Datel       scaffold_2:241249;       AACCAGATCCACCAGAGAGAGAGAGAGAGAAGAATT         oAS104       using Datel       scaffold_2:241249;       AACCAGATCCACCAGAGAGAGAGAGAGAAGAATT         HB4       CAPS markers       G/A       AAGATCAAGATCAAGAGAGAGAGAGAGAGAGAGAGAGAGA  |            | HiB3 CAPS markers         |                      |                                 |
| Justice       HiB3 CAPS markers<br>avig<br>oAS1002       ArG       CACACCTGATECTAATCTGGTATGCAC         HiB4 CAPS markers<br>oAS1039       using Ddel       scaffold_2:241249:<br>GACACCAGATCCACCAGATGGTCTGCT         HIB4 CAPS markers<br>oAS1040       using Ddel       scaffold_2:241249:<br>GACACCAGATCAACAGAGAGAGAGAGAGAGAAGAATT         HIB5 CAPS markers<br>oAS1040       using Ddel       AACCAGATCAACAGATCAACAGAGAGAGAGAGAAGAATT         HIB5 CAPS markers<br>oAS991       using Apol       CTCTGGAATTTCCCAATGCTCTG         HIB5 CAPS markers<br>oAS910       scaffold_2:249356;       CGGTTCAGCATTGACATAGAAGAGTGATGGG         HIB6 CAPS markers<br>oAS911       scaffold_2:232505;       TAGTGACATTGACATAGAAGAGTGATGGG         HIB7 dCAPS       scaffold_2:303580;       ACAAGTGGGCAGACAAAGCCACTCA         HIB7 dCAPS       G/A       CTTACCGTATCATTTTCAAGAACATGACAGA         oAS910       markers using Ddel       CTTACCGTATCATTTTCAAGAACATGACAGA         HIB7 dCAPS       G/A       CTTACCGTATCATTTTCAAGAACATGACAGA         oAS910       markers using Ddel       CTTACCGTATCATTTTCAAGAACATGACAGA         HIB7 dCAPS       G/A       CTCACTCTTGGTACATCAAAACTCC         HIB8 dCAPS       G/A       CTCACTCTTGGTACATCAAAACTCCAGAGA         GAS910       markers using Parl       TGGGATTGCGTGGATTTTGTT         GAS97       HiG1 CAPS markers       G/A       CTCACTGTGGCATTAGACACAGC  | oAS1001    | using NIaIII              | scaffold 2.203131.   | AGTCAGAAGCAGGACCAGAGAGTTGTC     |
| oAS1002       using Nall       CACACCTGATCCTATAATCTGGTATGCAC         oAS1039       using Dal       scaffold_2:241249;       AACCAGATCCACCAGATGGTCTGCT         oAS1040       using Dal       scaffold_2:241249;       AACCAGATCCACCAGATGGTCTGCT         oAS1040       using Dal       scaffold_2:249356;       CGGTTCAGTACTTAAGAAGATCGGGTT         oAS991       using Dal       scaffold_2:249356;       CGGTTCAGTACTTAAGAAGTCGGGTT         oAS992       using And       GTTCTGGAATTTCCCAATTGCTCTG         HIB6 CAPS markers       CT       GTTGTGACATTGACATAGAAGAGTGAGGGGGAGAGAAGAGTGGG         oAS917       using Alul       scaffold_2:2252505;       TAGTGACATTGACATAGGAAGAGCACTCA         oAS918       using Dale!       scaffold_2:303580;       ACAAGTGGGCAGACAAGCCACTCA         oAS916       markers using Dale!       cTTACCGTATCATTTTCAAGAACATGACAGA         oAS917       Using Psrl       scaffold_2:333608;       TCACCTCTTTGGTCACACTGCA         oAS918       HiB8 CAPS       GT       CTCAATGGTGCAGTTAGTCAAAACTGCAA         oAS919       markers using Dale!       scaffold_2:33608;       TCACTCTTTGGTCACACTGCA         oAS90       HiB3 CAPS       GT       CTCAATGGTGCAGTTAGCAAAACTCC         oAS90       HiB3 CAPS       GT       CTCAATGGTGCAGTTAGGCAAACTGCAA         oAS90  | 0/10/1001  | HiB3 CAPS markers         | A/G                  | norenomicenconcentorioritere    |
| John 2002       Uning Math       CAACCETORTECTATATECTORT         OAS1039       using Date!       scaffold_2:241249;       AACCAGATCCACCAGATGGTCTGCT         OAS1040       using Date!       AACCAGATCCACCAGATGGTCTGCT         OAS1040       using Date!       AACCAGATCCACCAGATGGTCTGCT         OAS1040       using Apol       CGGTTCAGTAGTATTAAGAAGATTCGGTT         OAS991       using Apol       GTTCTGGAATTTCCCAATGGTCTGG         HB5 CAPS markers       CC       CT         OAS910       using Apol       GTTGTGACATTGACATAGAAGAGTGATGGG         AS918       using Apol       GTGTGACTCTAGCATAGGAAGAGAGTGATGGG         AS919       using Apol       GTGTGACTCTAGCATAGGAAGAGTGATGGG         AS918       using Apol       GTGTGACTCTAGCATAGGACAAGGCACTCA         AS919       markers using Ddel       CTTACCGTATCATTTTCAAGAACATGACAGA         AAS910       markers using Ddel       CTTACCGTATCATTTTCAAGAACATGACAGA         CAP5_F       markers using Pstl       scaffold_2:363608;       TCACCTCTTGGTACACATGCA         AATGTGGTGCAGTATACATCAAGACACTCC       using Taql       scaffold_7:9135785;       CTCAATGGTGCATTTGGTACACTGCA         AAS978       HG1 CAPS       scaffold_7:9135785;       ACAGAATGGGACAATGGGACAATGGAACAGC       GAAGGGGACTAA         AAS8800       markers using Ddel   | o A \$1002 | using Mall                | AG                   |                                 |
| oAS1039         using Ddel<br>Wing Ddel         scaffold_2:241249;<br>GA         AACCAGATCCACCAGATGGTCTGCT           oAS1040         using Apol<br>using Apol<br>Wing Apol         scaffold_2:249356;<br>CGGTTCAGTACTTAAGAAGAGAGAGAGAGAGAGAGAGAGA   | 0A51002    |                           |                      | CACACCIDATCCIATAATCIDDIATOCAC   |
| aAS1039       Using Ddel       Scatfold 2:241249;       AACCAGATCCACCAGATGGTCTCT         aAS1040       using Ddel       AAGATCAAGATCAAGAGAGAGAGAGAGAGAAGAATT         HB5 CAPS markers       scatfold 2:249356;       CGGTTCAGTACTTTAAGAAGTCGGTT         aAS991       using Apol       GTTCTGGAATTTCCCAATGCTCTG         bB5 CAPS markers       scatfold 2:252505;       TAGTGACATTGACATAGAAGAGTGATGGG         aAS910       markers       scatfold 2:252505;       TAGTGACATTGACATAGAAGAGTGATGGG         aAS911       using Alul       scatfold 2:232350;       TAGTGACATTGACATAGAAGAGTGATGGG         aAS916       markers using Dael       GC       GTGTGACTCTAGCAATAGGAAGCACTCA         BB7 dCAPS       G/A       GTTACCGTATCATTAGAAGAGCACTCA         aAS910       markers using Dael       CTTACCGTATCATTTTTCAAGAACATGACAGA         aAS910       markers using Pstl       TGGATTGCGTGACTTTGGT         cAPS - F       markers using Pstl       TGGATTGCGTGCATTTGTT         cAPS79       HiG1 CAPS       G/T       CTCAATGGTGCATTTAGCACAACCTCC         using Taql       scatfold 7:9135785;       ACAAGAATGGGGACAATGGGAACAAGC         aAS808       markers using Ddel       GAATCGTGAAACGTAA         aAS809       markers using Ddel       GAATGGTGAAACGTAA         aAS809       markers using Ddel  | 4.01020    | HIB4 CAPS markers         | 55 11 2 241240       |                                 |
| AB1040       AAGATCAAGATCAAGAGAGAGAGAGAGAGAGAAGAATT         HB5 CAPS markers       cGGTTCAGTACTTAAGAAGAGAGAGAGAGAGAAGAATT         aAS991       using Apol       cGGTTCAGTACTTAAGAAGTCGGTT         HB5 CAPS markers       CT       GTCTGGAATTTCCCAATTGCTCG         aAS992       using Apol       GTCTGGAATTTCCCAATTGCTCGGT         HB6 CAPS markers       cG'C       GTGTGACTTAAGAAGAGTGATGGG         aAS917       using Alul       GTGTGACTCTAGCATAGGAAGAGAGAGAGAGAGAGAGAGA   | 0AS1039    | using Ddel                | scaffold_2:241249;   | AACCAGATCCACCAGATGGTCTGCT       |
| aAS1040     using Ddel     AAGATCAAGATCAAGAGAGAGAGAGAAGAATT       HIBS CAPS markers     scaffold 2:249356;     CGGTTCAGTACTTTAAGAAGAGAGAGAGAGAGAAGAATT       oAS991     using Apol     Scaffold 2:249356;     CGGTTCAGTACTTTAAGAAGATCAAGAGGAGAGAAGAAGTT       oAS991     using Apol     GTTCTGGAATTTCCCAATGCTTG       oAS910     using Apol     GTGTGACTTGACATAGAAGAGTGATGGG       oAS916     markers using Ddel     Scaffold 2:232505;     TAGTGACATTGACATAGAAGAGCACTCA       oAS916     markers using Ddel     Scaffold 2:303580;     ACAAGTGGGGCAGACAAGCCACTCA       HIB7 dCAPS     G/A     GTTACCGTATCATTTTCAAGAACATGACAGA       oAS910     markers using Ddel     CTTACCGTATCATTTTCAAGAACATGACAGA       HIB8 dCAPS     G/A     CTTACCGTATCATTTTCAAGAACATGACAGA       CAPS _R     markers using Psrl     TGGATTGCGTGCAGTTACATCAACTCTC       using Taql     scaffold 7:988899;     AATGTGGGGACAATGGATAACC       hIG2 dCAPS     G/A     AATGTGGGGACAATGGAACAACGC       using Taql     scaffold 7:9135785;     ACAAGAAGGGGACAATGGAACACGC       oAS800     markers using Ddel     GAATCGTGATATCGTATCTTCATTGGGAGAGAGAGAGAGA  |            | HiB4 CAPS markers         | G/A                  |                                 |
| HiBS CAPS markers       scaffold 2:249356 ;       CGGTTCAGTACTTTAAGAAGTTCGGTT         oAS991       using Apol       GTTCTGGAATTTCCCAATTGCTTG         oAS992       using Alol       GTTCTGGAATTTCCCAATTGCTCTG         HIBS CAPS markers       G/C       TAGTGACATTGACATAGAAGAGTGATGGG         oAS917       using Alul       scaffold_2:252505 ;       TAGTGACATTGACATAGAAGAGTGATGGG         oAS918       using Alul       GTGTGACTCTAGCATAAGGACATGACAGCC         oAS910       markers using Ddel       scaffold_2:303580 ;       ACAAGTGGGCAGACAAGCCACTCA         HiB7 dCAPS       G/A       CTTACCGTATCATTTTCAAGAACATGACAGA         HiB8 dCAPS       G/T       TCACCTCTTTGGTCACACTGCA         HiB8 dCAPS       G/T       TCACCTCTTGGCACACTGCA         HiB8 dCAPS       G/T       TGGATTGCGTGATTTGTT         oAS970       HiG1 CAPS markers       scaffold_7:9135785 ;       ACAAGGGGCACAATGGAACAGC         using Taql       scaffold_7:9135785 ;       ACAAGGAACAGGTGACAAGGCAATGGATAACC       HiG2 dCAPS         oAS808       markers using Ddel       scaffold_7:910575 ;       ACAAGGAGAACAGTTCGAGTATCCTATGACACTGGA         oAS800       markers using Xcml       scaffold_7:9253895 ;       ACAAGGTGAGAACAGTTCGAGGGAACAGTCCACTGGA         oAS811       markers using Xcml       scaffold_7:9106757 ;       AGCTGAGAGAAC  | oAS1040    | using <i>DdeI</i>         |                      | AAGATCAAGATCAAGAGAGAGAGAAGAATT  |
| oAS991       using Apol       scaffold 2:249356;       CGGTTCAGTACTTTAAGAAGTTCGGTT         oAS992       using Apol       GTTCTGGAATTTCCCAATTGCTTG         HBB CAPS markers       cACA       TAGTGACATTGACATAGAAGAGTGATGGG         oAS917       using Alul       scaffold_2:252505;       TAGTGACATTGACATAGAAGAGTGATGGG         oAS918       using Alul       scaffold_2:303580;       ACAAGTGGGCAGACAAGCCACTCA         HBB 7 (CAPS       GTACCGTACATTGACATGACAAGCACAAGCCACTCA         HBB 7 (CAPS       GA       CTTACCGTATCATTTTCAAGAACATGACAGA         oAS910       markers using Ddel       CTTACCGTATCATTTTGAGAACATGACAGA         GAS910       markers using Ddel       CTTACCGTATCATTTGGTCACACTGCA         HBB 4CAPS       GA       GAT         GAS98       markers using Pstl       TCGCATTGGTGCATTTAGGCAACAGC         using Taql       scaffold_7:9185785;       ACAAGAATGGGGACAATGGAACAAGC         using Taql       scaffold_7:9135785;       ACAAGAAATGGGGAACAAGGCAACAGC         oAS809       markers using Ddel       scaffold_7:9135785;       ACAAGAAACTGTGAATATGGAACAAGGAAGAGTAT         oAS800       markers using Ddel       scaffold_7:9106757;       AGCTGGAGAACAGGTCACATGGAACAGC         oAS810       markers using Zonl       scaffold_7:9253895;       GAAAGGGAGATTA         GAAAGGGACTTAA <td></td> <td>HiB5 CAPS markers</td> <td></td> <td></td>   |            | HiB5 CAPS markers         |                      |                                 |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | oAS991     | using Apol                | scaffold_2:249356;   | CGGTTCAGTACTTTAAGAAGTTCGGTT     |
| oAS992         using Apol         GTTCTGGAATTTCCCAATTGCTCTG           HiB6 CAPS markers         scaffold_2:252505;         TAGTGACATTGACATAGAAGAGTGATGGG           OAS918         using Alul         GTC           oAS918         using Alul         GTGTGACTCTAGCATAGAAGAGTGATGGG           oAS918         using Alul         GTGTGACTCTAGCATAAGGTATTGTAAGCTC           HiB7 dCAPS         GTA         GTACCGTAGCATAGGAACAAGCCACTCA           HiB7 dCAPS         Scaffold_2:303580;         ACAAGTGGGCAGACAAGCCACTCA           HiB8 dCAPS         GT         CAP5_F         markers using Pstl         CTACCTCTTTGGTCACACTGCA           CAP5_F         markers using Pstl         GT         TGGATTGCGTGATTTTGTT           oAS797         HiG1 CAPS markers         GA         CTCACTGTGGCAGTTACATCAAACCTCC           using Taql         scaffold_7:886399;         CTCAATGGGGACAATGGAACAGC           oAS808         HiG2 dCAPS         GA         AATGTGGGGACAATGGAACAGC           using Taql         scaffold_7:9135785;         ACAAGAATGGGGAACAATGGAACAGC           oAS808         markers using Ddel         GAATCGTGAACAGGAACAGTTCTGTGTAACC           HiG3 dCAPS         A/G         AG           oAS810         markers using Xeml         scaffold_7:913578;           AGCTGAGGAACAGGTTCGAGGAACAGTTCTGGGACACAGGTT  |            | HiB5 CAPS markers         | C/T                  |                                 |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | oAS992     | using Apol                |                      | GTTCTGGAATTTCCCAATTGCTCTG       |
| oAS917       using Alul       scaffold_2:25205;       TAGTGACATTGACATAGAAGAGTGATGGG         oAS918       using Alul       GTGTGACTCTAGCATAAGGTATTGTAAGCTC         oAS916       markers using Ddel       scaffold_2:303580;       ACAAGTGGGCAGACAAGCCACTCA         oAS910       markers using Ddel       G/A       CTTACCGTATCATTTTCAAGAACATGACAGA         oAS910       markers using Ddel       CTTACCGTATCATTTTCAAGAACATGACAGA         CAP5_F       markers using Psrl       Scaffold_2:303680;       TCACCTCTTTGGTCACACTGCA         CAP5_R       markers using Psrl       CTCACTGGTGCAGTTACATCAAACTCTC         using Taql       scaffold_7:8868399;       CTCAATGGTGCAGTTACATCAAACTCTC         using Taql       scaffold_7:9135785;       ACAAGGAGACAAGGGAACAAGC         HiG2 dCAPS       A/G       A/G         oAS808       markers using Ddel       scaffold_7:9106757;       AGCTGGAGAACAGGTCATTGGTGTCACT         oAS809       markers using Xcml       scaffold_7:9253895;       AAGGCTGAACAGCTCAA         oAS810       markers using Xcml       scaffold_7:9253895;       AAGGCTGAACAGGTCCAGGAGTAT         oAS810       markers using Xcml       scaffold_7:9253895;       AAGGCTAAACTCCAGGGTCGA         oAS81249       HiG4 Indel markers       Scaffold_7:9253895;       AAGGCTAAACTCCAGGGTCGA         oAS806 <td></td> <td>HiB6 CAPS markers</td> <td></td> <td></td>   |            | HiB6 CAPS markers         |                      |                                 |
| ABJON       HiB6 CAPS markers       G/C         oAS918       using Alul       GTGTGACTCTAGCATAAGGTATTGTAAGCTC         HB7 dCAPS       G/A       G/A         oAS916       markers using Ddel       scaffold_2:303580;       ACAAGTGGGCAGACAAGCCACTCA         OAS910       markers using Ddel       G/A       CTTACCGTATCATTTTCAAGAACATGACAGA         HB8 dCAPS       G/T       CAP5 F       markers using Pstl       scaffold_2:363608;       TCACCTCTTTGGTCACACTGCA         CAP5 R       markers using Pstl       scaffold_2:363608;       TCACCTCTTTGGTCACACTGCA       G/A         oAS797       HiG1 CAPS markers       G/A       G/A       AATGTGGTGCAGTTACATCAAACTCTC         using Taql       scaffold_7:9135785;       ACAAGAATGGGGACAATGGCAACAGC       HiG2 dCAPS         oAS808       markers using Ddel       GAATCGTGATATCGTATCTCTCATTG         oAS809       markers using Ddel       GAATCGTGAATATCGTATCTGTGTCACT         HiG2 dCAPS       A/G       GAATCGTGAAACGCTAA         oAS809       markers using Ddel       GAATCGTGAATTGCATCCATGGAAGTAT         oAS811       markers using Xcml       TTTTTGGAATTGCATCCATGGAGTATA         GGACTAACGCTAA       GGAGTAAGCTAA       ACCCCAGGGTTCG         oAS1249       HiG4 Indel markers       Scaffold_7:9374725;       CGTGCAGAGA  | oAS917     | using Alul                | scaffold 2:252505 :  | TAGTGACATTGACATAGAAGAGTGATGGG   |
| oAS918       using Alul       GTGTGACTCTAGCATAAGGTATTGTAAGCTC         HiB7 dCAPS       scaffold 2:303580;       ACAAGTGGGCAGACAAGCCACTCA         HiB7 dCAPS       G/A       CTTACCGTATCATTTTCAAGAACATGACAGA         oAS910       markers using Ddel       CTTACCGTATCATTTTCAAGAACATGACAGA         HiB8 dCAPS       G/A       CTACCGTCTTTGGTCACACTGCA         CAP5_F       markers using Pstl       scaffold_2:363608;       TCACCTCTTTGGTCACACTGCA         OAS797       HiG1 CAPS markers       G/A       CTCAATGGTGCAGTTACATCAAACATCTCC         using Taql       scaffold_7:8868399;       OAS798       AATGTGGGTGCAATTAGGCAACAGC         using Taql       scaffold_7:9135785;       ACAAGAATGGGGACAATGGGAACAGC         using Taql       scaffold_7:9135785;       ACAAGAATGGGGACAATGGATAACC         HiG2 dCAPS       A/G       GAATCGTGATATCGTATCTCATTG         oAS808       markers using Ddel       scaffold_7:910577;       AGCTGGAGAACAGTTCTGTGTCACT         HiG3 dCAPS       A/G       GAAGGGAGTTA       GAAGCTAAACTCCAGGGTTCGA       GAAGGGAGTTA         oAS810       markers using Zcml       scaffold_7:9106757;       AGCTGGAGAACAGTCCATGGAACAGTAT       GAAGCTCAAGGTAA         oAS1249       HiG4 Indel markers       scaffold_7:923895;       GAAGGGAGTTA       GGAGTTAAGCTAA       GCTCCAGGGTCCAGGGCCAATGGAACAGTTCGA  |            | HiB6 CAPS markers         | G/C                  |                                 |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | 045918     | using Alul                | 0,0                  | GTGTGACTCTAGCATAAGGTATTGTAAGCTC |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | 0110/10    | HiB7 dCAPS                |                      | Gibionerennisennisennisene      |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | 04\$016    | markars using Ddel        | conffold 2.202580.   |                                 |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | 0A3910     |                           | scalloid_2.505580,   | ACAAOTOOOCAOACAAOCCACTCA        |
| OAS910       markers using Date       CTTACCGTATCATTTTCAAGAACATGACAGA         HiB8 dCAPS       scaffold_2:363608;       TCACCTCTTTGGTCACACTGCA         CAP5_R       markers using Pstl       G/T         oAS797       HiG1 CAPS markers       G/T         using Taql       scaffold_7:8868399;       CTCAATGGTGCAGTTACATCAAACACTCC         using Taql       scaffold_7:8868399;       AATGTGGTGCAGTTACATCAAACACGC         oAS798       HiG1 CAPS markers       G/A       AATGTGGTGCAATGGGAACAAGC         using Taql       scaffold_7:9135785;       ACAAGAATGGGGAACAATGGATAACC         oAS808       markers using Ddel       scaffold_7:9135785;       ACAAGAATGGGGAACAAGTTCTGTATCCTCATTTG         oAS809       markers using Zcml       scaffold_7:9106757;       AGCTGGAGAACAGTTCTGTGTCACT         HiG3 dCAPS       A/G       TTTTTGGAATTGCATCCATGGAAGTAT         oAS810       markers using Xcml       scaffold_7:9253895;       AGGCTGAAACCTCCAGGGTTCGA         oAS811       markers using Xcml       Scaffold_7:9253895;       AAGCTAAACCTCCAGGGTTCGA         GAAAGGGAGTTA       GGAGTAAGCTAAA       GGAGTAAGCTAA       AGCTCAGAGGTGCAGTGG         oAS1249       HiG4 Indel markers       Scaffold_7:9374725;       CGTTGCAGAGAGCAGTTA         GAS806       using Xmnl       scaffold_7:9374725;       CGTTG   | 4 0010     | HIB/ dCAPS                | U/A                  |                                 |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | 0AS910     | markers using Dael        |                      | CITACCGTATCATTITCAAGAACATGACAGA |
| $\begin{array}{c} CAP5\_F & markers using Pstl \\ HiB8 dCAPS & G/T \\ CAP5\_R & markers using Pstl \\ oAS797 & HiG1 CAPS markers \\ using Taql \\ \hline oAS798 & HiG1 CAPS markers \\ using Taql \\ \hline oAS798 & markers using Ddel \\ \hline HiG2 dCAPS \\ oAS808 & markers using Ddel \\ \hline oAS809 & markers using Neml \\ \hline HiG3 dCAPS \\ oAS810 & markers using Neml \\ \hline oAS811 & markers using Neml \\ \hline oAS1249 & HiG4 Indel markers \\ \hline oAS1249 & HiG4 Indel markers \\ \hline oAS1250 & HiG4 Indel markers \\ \hline oAS806 & using Xmnl \\ \hline oAS806 & using Xmnl \\ \hline oAS1250 & HiG4 Indel markers \\ \hline oAS807 & using Xmnl \\ \hline oAS806 & using Xmnl \\ \hline oAS807 & using Xmnl \\ \hline oACAPS & markers \\ \hline oCT $ |            | HiB8 dCAPS                |                      |                                 |
| $\begin{array}{c c} HiBs dCAPS & G/T \\ \hline CAP5\_R & markers using Pstl & TGGATTGCGTGATTTTGTT \\ oAS797 & HiG1 CAPS markers & caffold_7:8868399 ; \\ \hline oAS798 & HiG1 CAPS markers & G/A & AATGTGGTGCAGTTACATCAAACTCTC \\ \hline using Taql & & \\ using Taql & & \\ HiG2 dCAPS & & \\ markers using Ddel & scaffold_7:9135785 ; \\ ACAAGAATGGGGACAATGGATAACC & \\ HiG2 dCAPS & & \\ HiG3 dCAPS & & \\ A/G & & \\ AAGCTGGAGAACAGTTCTGTGTCACT & \\ HiG3 dCAPS & & \\ A/G & & \\ AAGCTGGAGAACAGTTCTGTGTCACT & \\ HiG3 dCAPS & & \\ A/G & & \\ markers using Xcml & & \\ scaffold_7:9106757 ; & \\ AGCTGGAGAACAGTTCTGTGTCACT & \\ HiG3 dCAPS & & \\ A/G & & \\ AAGCTAAACTCCAGGGTTC & \\ GAAS811 & markers using Xcml & & \\ Scaffold_7.9253895; & \\ AAGCTAAACTCCAGGGTTCGA & \\ AGCTAAACTCCAGGGTTCGA & \\ GGAG/TAAGCTAA & \\ ACTCCAGGGTTC & \\ GAAGGGAGTAA & \\ ACTCCAGGGTTC & \\ GAANNNNNNNNN & \\ TGTTTACTTGGG & TGTTTTACTTGGGTGCAGTTGG & \\ HiG5 CAPS markers & \\ OAS806 & using Xmnl & \\ Scaffold_7:9374725 ; & \\ CGTTGCAGAGAGGAGTTA & \\ HiG5 CAPS markers & \\ OAS807 & using Xmnl & \\ Scaffold_7:9569807 ; & \\ HiG6 CAPS markers & \\ GOT_F & using Hinfl & \\ HiG6 CAPS markers & \\ C/T & \\ \hline GOT_R & using Hinfl & \\ TGGATACGGTGCCATAGGGGCCTATGGTCC & \\ TGGATACAGGTGGCCTATGGTGCC & \\ TGGATACAGGTGGCCTATGGTGCC & \\ AAGGATGCTGTCAGGTGCC & \\ AAGGATGCTGTCAGCTGCCACGATTGGGTAATA & \\ \hline HiG6 CAPS markers & \\ C/T & \\ \hline GOT_R & using Hinfl & \\ TGGATACAGGTGCCCACGATGGGCCTATGGTC & \\ TGGATACAGGTGCCCACGATGGTCC & \\ \hline AAGGATGCTGTCAGGTGCCCTATGGTCC & \\ \hline AAGGATGCTGCCACGGTGCCCTATGGTGC & \\ \hline TGGATACAGGTGGCCTATGGTGCC & \\ \hline CT & \\ \hline GOT_R & using Hinfl & \\ TGTACTGGTGCCACGGTGCCCACGATGGTC & \\ \hline TGGATACAGGTGGCCCTATGGTCC & \\ \hline TGGATACAGGTGCCCTATGGTCC & \\ \hline TGGATACAGGTGGCCCTATGGTCC & \\ \hline TGGATACAGGTGGCCCTATGGTCC & \\ \hline TGGATACAGGTGCCCTATGGTC & \\ \hline TGGATACAGGTGGCCCTATGGTC & \\ \hline TGGATACAGGTGGCCCTATGGTC & \\ \hline TGGATACAGGTGCCCTATGGTC & \\ \hline TGGATACAGGTGCCCTATGGTC & \\ \hline TGGATACAGGTGCCCTATGGTC & \\ \hline TGGATACAGGTGCCCTATGGTC & \\ \hline TGGATACAGGTGCCCTAT$   | CAP5_F     | markers using <i>Pst1</i> | scaffold_2:363608;   | TCACCTCTTTGGTCACACTGCA          |
| $\begin{array}{c} CAP5\_R & markers using Pstl & TGGATTGGTGATTTGTT \\ \hline oAS797 & HiG1 CAPS markers \\ using Taql & scaffold\_7:8868399; \\ \hline oAS798 & HiG1 CAPS markers & G/A & AATGTGGTGCAGTTACATCAAACTCTC \\ \hline using Taql & HiG2 dCAPS \\ oAS808 & markers using Ddel & scaffold\_7:9135785; & ACAAGAATGGGGACAATGGATAACC \\ \hline HiG2 dCAPS & A/G & \\ \hline oAS809 & markers using Ddel & GAATCGTGATATCGTATCTCTCATTTG \\ \hline HiG3 dCAPS & A/G & \\ \hline oAS810 & markers using Xcml & scaffold\_7:9106757; & AGCTGGAGAACAGTTCTGTGTCACT \\ \hline HiG3 dCAPS & A/G & \\ \hline oAS811 & markers using Xcml & scaffold\_7.9253895; & \\ \hline oAS1249 & HiG4 Indel markers & \\ \hline Scaffold\_7.9253895; & \\ \hline oAS1249 & HiG4 Indel markers & \\ \hline Scaffold\_7.9253895; & \\ \hline GAAAGGGAGTTA & \\ \hline GAANNNNNNNN & \\ TGTTTTACTTGGG & TGTTTTACTTGGGTGCAGTTGG \\ \hline HiG5 CAPS markers & \\ \hline oAS806 & using Xnnl & \\ \hline oAS807 & using Xnnl & \\ \hline OAS807 & using Xnnl & \\ \hline HiG6 CAPS markers & \\ \hline OAS807 & using Xnnl & \\ \hline OAS807 & using Xnnl & \\ \hline HiG6 CAPS markers & \\ \hline OAS807 & using Xnnl & \\ \hline OAS808 & $  |            | HiB8 dCAPS                | G/T                  |                                 |
| oAS797       HiG1 CAPS markers<br>using Taql       scaffold_7:8868399;       CTCAATGGTGCAGTTACATCAAAACTCTC         oAS798       HiG1 CAPS markers<br>using Taql       scaffold_7:8868399;       AATGTGGTGCATTTAGGCAACAGC         HiG2 dCAPS       markers using Ddel       scaffold_7:9135785;       ACAAGAATGGGGACAATGGATAACC         OAS808       markers using Ddel       GAATCGTGATATCGTATCTCATTTG         HiG2 dCAPS       A/G       GAATCGTGATATCGTATCTCATTTG         oAS809       markers using Ddel       GAATCGTGAAACAGTTCTGTGTCACT         HiG3 dCAPS       A/G       TTTTTGGAATTGCATCATGGAAGTAT         oAS810       markers using Xcml       scaffold_7:9106757;       AGCTGGAGAACAGTTCTGTGTCACT         oAS811       markers using Xcml       scaffold_7:9253895;       AAGGTAAACTCAAGGGAGTTA         oAS1249       HiG4 Indel markers       scaffold_7:9253895;       AAGGTAAGCTAA         GGAG/TAAGCTAA       ACCCCAGGGTTC       AAGCTCAAGGTAGC         oAS806       using Xnn1       scaffold_7:9374725;       CGTTGCAGAGAGAGAGTTAA         HiG5 CAPS markers       A/C       GTCGCTGCCACGATTGGGTAATA         oAS807       using Xnn1       scaffold_7:9569807;       AAGGATGCTGCAGCAGTTGAGCTTCAGGTGAATA         HiG6 CAPS markers       G/T       GTCGCTGCCACGATTGGGTGCAGTGCCTTCTTG         G07_F       using H  | CAP5_R     | markers using PstI        |                      | TGGATTGCGTGATTTTGTT             |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | oAS797     | HiG1 CAPS markers         |                      | CTCAATGGTGCAGTTACATCAAACTCTC    |
| oAS798       HiG1 CAPS markers<br>using <i>Taql</i> G/A       AATGTGGGTGCATTTAGGCAACAGC         HiG2 dCAPS       scaffold_7:9135785;       ACAAGAATGGGGACAATGGATAACC         oAS808       markers using <i>Ddel</i> GAATCGTGATATCGTATCTCTCATTGG         HiG2 dCAPS       A/G       GAATCGTGATATCGTATCTCTCATTGG         oAS809       markers using <i>Ddel</i> GAATCGTGAGAACAGTTCTGTGTCACT         HiG3 dCAPS       scaffold_7:9106757;       AGCTGGAGAACAGTTCTGTGTCACT         oAS811       markers using <i>Xcml</i> TTTTTGGAATTGCATCCATGGAAGTAT         oAS1249       HiG4 Indel markers       Scaffold_7.9253895;       AAGCTAAACTCCAGGGTTCGA         GAAAGGGAGTTA       GGAG/TAAGCTAA       ACTCCCAGGGTTC       AAGCTAAACTCCAGGGTTCGA         oAS1249       HiG4 Indel markers       TGCAGTTGG       TGTTTACTTGGGTGCAGTTGG         oAS1250       HiG4 Indel markers       Scaffold_7:9374725;       CGTTGCAGAGAGAGCTTTAATAGAATTTC         oAS806       using <i>Xmnl</i> Scaffold_7:9374725;       CGTTGCAGAGAGAGCTTTAATAGAATTTC         HiG5 CAPS markers       A/C       GTCGCTTGCCACGATTGGGTAATA         oAS807       using <i>Xmnl</i> GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       C/T       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       C/T       GTGGATACAGGTGCTGTCAGCTGCTCTTG <td></td> <td>using TaqI</td> <td>scaffold_7:8868399;</td> <td></td>   |            | using TaqI                | scaffold_7:8868399;  |                                 |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | oAS798     | HiG1 CAPS markers         | G/A                  | AATGTGGTGCATTTAGGCAACAGC        |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  |            | using TaqI                |                      |                                 |
| oAS808       markers using Ddel       scaffold_7:9135785;       ACAAGAATGGGGGACAATGGATAACC         HiG2 dCAPS       A/G       GAATCGTGATATCGTATCTCTCATTTG         oAS809       markers using Ddel       GAATCGTGATATCGTATCTCTCATTTG         HiG3 dCAPS       scaffold_7:9106757;       AGCTGGAGAACAGTTCTGTGTCACT         HiG3 dCAPS       A/G       TTTTTGGAATTGCATCCATGGAAGATAT         oAS811       markers using Xcml       Scaffold_7.9253895;       GAAGGGAGTTA         oAS1249       HiG4 Indel markers       Scaffold_7.9253895;       GAAGGTAAGCTAA         GGAG/TAAGCTAA       GGAG/TAAGCTAA       ACTCCAGGGTTC G         GANNNNNNNNN       TGTTTTACTTGGG       TGTTTTACTTGGG         oAS1250       HiG4 Indel markers       TGCAGTTGG       TGTTTTACTTGGGGTGCAGTTGG         HiG5 CAPS markers       GA/G       GTCGCTTGCAGAGAGCTTTAATAGAATTTC         oAS806       using Xmnl       scaffold_7:9374725;       CGTTGCAGAGAGCTTTAATAGAATTTC         HiG6 CAPS markers       A/C       GTCGCTTGCCACGATTGGGTAATA       MiG6 CAPS markers         G07_F       using Xmnl       GTCGCTTGCCACGATGGCGCTATGGTGTC       AAGGATGCTGTCAGGTGTC         n.a. not       applicable       Scaffold_7:9569807;       AAGGATACAGGTGCCTATGGTGTC  |            | HiG2 dCAPS                |                      |                                 |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $  | oAS808     | markers using <i>Ddel</i> | scaffold 7:9135785 : | ACAAGAATGGGGACAATGGATAACC       |
| oAS809     markers using Ddel     GAATCGTGATATCGTATCTCTCATTTG       HiG3 dCAPS     scaffold_7:9106757 ;     AGCTGGAGAACAGTTCTGTGTCACT       HiG3 dCAPS     A/G     TTTTTGGAATTGCATCCATGGAAGTAT       oAS810     markers using Xcml     A/G       oAS811     markers using Xcml     TTTTTGGAATTGCATCCATGGAAGTAT       oAS1249     HiG4 Indel markers     scaffold_7.9253895;     AAGCTAAACTCCAGGGTTCGA       GAAAGGGAGTTA     GGAGGTAAAGCTAAA     ACTCCAGGGTTC       GANNNNNNNNN     TGTTTTACTTGGG     TGTTTTACTTGGGTGCAGTTGG       oAS1250     HiG4 Indel markers     scaffold_7:9374725 ;     CGTTGCAGAGAGGCTTTAATAGAATTC       oAS806     using Xmnl     scaffold_7:9374725 ;     CGTTGCAGAGAGAGCTTTAATAGAATTC       HiG5 CAPS markers     A/C     GTCGCTTGCCACGATTGGGTAATA       oAS807     using Xmnl     scaffold_7:9374725 ;     CGTTGCCACGATTGGGTAATA       HiG6 CAPS markers     A/C     GTCGCTTGCCACGATTGGGTAATA       HiG6 CAPS markers     C/T     GGAGTGCCTATGGTGCAGTGCCTTCTTG       G07_F     using Hinfl     scaffold_7:9569807 ;     AAGGATGCTGTCAGCTGCCTATGGTGTC       n.a. not     applicable     K     K     K  | 0110000    | HiG2 dCAPS                | A/G                  |                                 |
| oAS800       Indicest using Data       ONATEGUTOATATEGUTATETETETEATTIC         oAS810       markers using Xcml       scaffold_7:9106757 ;       AGCTGGAGAACAGTTCTGTGTCACT         oAS811       markers using Xcml       TTTTTGGAATTGCATCCATGGAAGTAT         oAS1249       HiG4 Indel markers       scaffold_7.9253895;       AAGCTAAACTCCAGGGTTCGA         GAAAGGGAGTTA       GGAG/TAAGCTAA       AGCTCCAGGGTTCGA         GAANNNNNNNNNN       TGTTTACTTGGG       AGCTTGCAGGTGCAGTTGG         oAS1250       HiG4 Indel markers       TGCAGTTGG       TGTTTTACTTGGGG         oAS806       using Xmnl       scaffold_7:9374725 ;       CGTTGCAGAGAGCTTTAATAGAATTC         oAS807       using Xmnl       scaffold_7:9374725 ;       CGTTGCCACGATTGGGTAATA         HiG6 CAPS markers       A/C       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       C/T       GGATGCTGTCAGCTGCCTTCTTG         G07_R       using Hinfl       scaffold_7:9569807 ;       AAGGATGCTGTCAGCTGCCTATGGTGTC         n.a. not       applicable       Japplicable       Japplicable       Japplicable  | 048809     | markers using Ddal        |                      | GAATCGTGATATCGTATCTCTCATTTG     |
| oAS810       markers using Xcml       scaffold_7:9106757 ;       AGCTGGAGAACAGTTCTGTGTCACT         oAS811       markers using Xcml       TTTTTGGAATTGCATCCATGGAAGTAT         oAS1249       HiG4 Indel markers       scaffold_7.9253895;       AAGCTAAACTCCAGGGTTCGA         GAAAGGGAGTTA       GGAGATAAGCTAA       ACTCCAGGGTTC       AAGCTAAACTCCAGGGTTCGA         oAS1249       HiG4 Indel markers       scaffold_7.9253895;       AAGCTAAACTCCAGGGTTCGA         GGAGATAAGCTAA       ACTCCAGGGGTCC       GANNNNNNNNN         TGTTTTACTTGGG       GANNNNNNNNNN         TGTTTTACTTGGGTGCAGTTGG       HiG5 CAPS markers         oAS806       using Xmnl       scaffold_7:9374725 ;         CGTTGCAGAGAGAGCTTTAATAGAATTTC       HiG6 CAPS markers         oAS807       using Xmnl       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       C/T         G07_F       using Hinfl       scaffold_7:9569807 ;         AAGGATGCTGTCAGGTGCCTATGGTGTC       n.a. not         applicable       Jinfl  | 0/10007    | HiG3 dCAPS                |                      | Simileoromineormeteremitio      |
| oA3810       Intakers using Xcml       scaffold_7.9100/37 , AGCFOOADAACAOFTCFOTOTCACT         oAS811       markers using Xcml       TTTTTGGAATTGCATCCATGGAAGTAT         oAS1249       HiG4 Indel markers       scaffold_7.9253895;<br>GAAAAGGAGTTA<br>GGAG/TAAGCTAA<br>ACTCCAGGGTTC<br>GANNNNNNNN<br>TGTTTTACTTGGG       AAGCTAAACTCCAGGGTTCGA         oAS1250       HiG4 Indel markers       TGCAGTTGG       TGTTTTACTTGGGTGCAGTTGG         oAS806       using Xmnl       scaffold_7:9374725 ;<br>HiG5 CAPS markers       CGTTGCAGAGAGAGCTTTAATAGAATTTC         oAS807       using Xmnl       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       A/C       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       C/T       GGATACAGTGCTGTCAGCTGCCTTCTTG         G07_F       using Hinfl       scaffold_7:9569807 ;<br>HiG6 CAPS markers       AAGGATGCTGTCAGCTGCCTATGGTGTC         n.a. not       applicable       J       TGGATACAGGTGGCCTATGGTGTC   | 045810     | morkors using VamI        | ageffold 7,0106757.  | ACCTCCACAACACTTCTCTCTCTCACT     |
| ndfs dCAFS     A/G       oAS811     markers using Xcml     TTTTTGGAATTGCATCCATGGAAGTAT       oAS1249     HiG4 Indel markers     scaffold_7.9253895;<br>GAAAGGGAGTTA<br>GGAG/TAAGCTAA<br>ACTCCAGGGTTC<br>GANNNNNNNN<br>TGTTTTACTTGGG     AAGCTAAACTCCAGGGTTCGA       oAS1250     HiG4 Indel markers     TGCAGTTGG     TGTTTTACTTGGGTGCAGTTGG       oAS806     using Xmnl     scaffold_7:9374725;<br>A/C     CGTTGCAGAGAGAGTATA       oAS807     using Xmnl     scaffold_7:9374725;<br>A/C     CGTTGCCACGATTGGGTAATA       oAS807     using Xmnl     GTCGCTTGCCACGATTGGGTAATA       HiG6 CAPS markers     A/C     GTCGCTTGCCACGATTGGGTAATA       G07_F     using Hinfl     scaffold_7:9569807;<br>HiG6 CAPS markers     AAGGATGCTGTCAGCTGCCTTCTTG       G07_R     using Hinfl     TGGATACAGGTGGCCTATGGTGTC     n.a. not<br>applicable   | 0A3010     |                           | scariold_7.9100757;  | AUCIUDAUAACAUTICIUTUTCACI       |
| oAS811       markers using Xcml       ITTTGGAATTGCATCCATGGAAGTAT         oAS1249       HiG4 Indel markers       scaffold_7.9253895;<br>GAAAGGGAGTTA<br>GGAG/TAAGCTAA<br>ACTCCAGGGTTC<br>GANNNNNNNN<br>TGTTTTACTTGGG       AAGCTAAACTCCAGGGTTCGA         oAS1250       HiG4 Indel markers       TGCAGTTGG       TGTTTTACTTGGGTGCAGTTGG         oAS806       using Xmnl       TGCAGTTGG       TGTTGCAGAGAGAGCTTTAATAGAATTTC         HiG5 CAPS markers       scaffold_7:9374725;       CGTTGCAGAGAGAGCTTTAATAGAATTTC         HiG5 CAPS markers       A/C       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       scaffold_7:9569807;       AAGGATGCTGTCAGCTGCCTTCTTG         G07_F       using Hinf1       scaffold_7:9569807;       AAGGATGCTGTCAGCTGCCTTCTTG         G07_R       using Hinf1       TGGATACAGGTGGCCTATGGTGTC       n.a. not         applicable       Ling Hinf1       TGGATACAGGTGGCCTATGGTGTC       Ling Applicable   | 40011      | HIGS dCAPS                | A/O                  |                                 |
| oAS1249       HiG4 Indel markers       scatfold_/.9253895;<br>GAAAGGGAGTTA<br>GGAAGGGAGTTA<br>GGAAGGGAGTTA<br>GGAG/TAAGCTAA<br>ACTCCAGGGTTC<br>GANNNNNNNN<br>TGTTTTACTTGGG       AAGCTAAACTCCAGGGGTTCGA         oAS1250       HiG4 Indel markers       TGCAGTTGG       TGTTTTACTTGGGTGCAGTTGG         oAS1250       HiG4 Indel markers       TGCAGTTGG       TGTTTTACTTGGGTGCAGTTGG         oAS806       using Xmn1       scaffold_7:9374725;       CGTTGCAGAGAGCTTTAATAGAATTTC         HiG5 CAPS markers       A/C       OTCGCTTGCCACGATTGGGTAATA         oAS807       using Xmn1       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       Scaffold_7:9569807;       AAGGATGCTGTCAGCTGCCTTCTTG         G07_F       using Hinf1       scaffold_7:9569807;       AAGGATACAGGTGGCCTATGGTGTC         n.a. not       applicable       January Context of the state of t   | 0A5811     | markers using XcmI        | SC 11 7 0252005      | IIIIIGGAAIIGCAICCAIGGAAGIAI     |
| GAAAGGGAGTTA         GGAAGGGAGTTA         GGAG/TAAGCTAA         ACTCCAGGGTTC         GANNNNNNNN         TGTTTTACTTGGG         oAS1250       HiG4 Indel markers         TGCAGTTGG       TGTTTTACTTGGGTGCAGTTGG         HiG5 CAPS markers       GCGTTGCAGAGAGCTTTAATAGAATTTC         HiG5 CAPS markers       A/C         oAS806       using Xmnl         scaffold_7:9374725 ;       CGTTGCAGAGAGCTTTAATAGAATTTC         HiG5 CAPS markers       A/C         oAS807       using Xmnl         GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       GTCGCTGTCAGCTGCCAGCTGCCTTCTTG         HiG6 CAPS markers       C/T         G07_F       using Hinfl       scaffold_7:9569807 ;         AAGGATGCTGTCAGCTGCCTATGGTGTC       n.a. not         applicable       Infl   | oAS1249    | HiG4 Indel markers        | scaffold_7.9253895;  | AAGCTAAACTCCAGGGTTCGA           |
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| ACTCCAGGGTTC<br>GANNNNNNN<br>TGTTTTACTTGGG<br>oAS1250 HiG4 Indel markers TGCAGTTGG TGTTTTACTTGGGTGCAGTTGG<br>HiG5 CAPS markers scaffold_7:9374725 ; CGTTGCAGAGAGAGCTTTAATAGAATTTC<br>HiG5 CAPS markers A/C<br>OAS807 using Xmnl GTCGCTTGCCACGATTGGGTAATA<br>HiG6 CAPS markers<br>G07_F using Hinfl scaffold_7:9569807 ; AAGGATGCTGTCAGCTGCCTTCTTG<br>HiG6 CAPS markers C/T<br>G07_R using Hinfl TGGATACAGGTGGCCTATGGTGTC<br>n.a. not<br>applicable  |            |                           | GGAG/TAAGCTAA        |                                 |
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| oAS1250       HiG4 Indel markers       TGCAGTTGG       TGTTTTACTTGGGTGCAGTTGG         HiG5 CAPS markers       scaffold_7:9374725 ;       CGTTGCAGAGAGCTTTAATAGAATTTC         oAS806       using XmnI       scaffold_7:9374725 ;       CGTTGCAGAGAGCTTTAATAGAATTTC         oAS807       using XmnI       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       A/C       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       C/T       AAGGATGCTGTCAGCTGCCTTCTTG         G07_F       using Hinf1       scaffold_7:9569807 ;       AAGGATGCTGTCAGCTGCCTTCTTG         G07_R       using Hinf1       TGGATACAGGTGGCCTATGGTGTC         n.a. not       applicable       TGGATACAGGTGGCCTATGGTGTC  |            |                           | TGTTTTACTTGGG        |                                 |
| HiG5 CAPS markers       scaffold_7:9374725 ;       CGTTGCAGAGAGCTTTAATAGAATTTC         HiG5 CAPS markers       A/C         oAS807       using XmnI       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       GTCGCTTGCCACGATTGGGTAATA         G07_F       using HinfI       scaffold_7:9569807 ;         HiG6 CAPS markers       C/T         G07_R       using HinfI         n.a. not       applicable   | oAS1250    | HiG4 Indel markers        | TGCAGTTGG            | TGTTTTACTTGGGTGCAGTTGG          |
| oAS806       using Xmn1       scaffold_7:9374725 ;       CGTTGCAGAGAGCTTTAATAGAATTTC         HiG5 CAPS markers       A/C         oAS807       using Xmn1       GTCGCTTGCCACGATTGGGTAATA         HiG6 CAPS markers       GTCGCTTGCCACGATTGGGTAATA         G07_F       using Hinf1       scaffold_7:9569807 ;         HiG6 CAPS markers       C/T         G07_R       using Hinf1         n.a. not       applicable   |            | HiG5 CAPS markers         |                      |                                 |
| HiG5 CAPS markers     A/C       oAS807     using XmnI     GTCGCTTGCCACGATTGGGTAATA       HiG6 CAPS markers     HiG6 CAPS markers       G07_F     using HinfI     scaffold_7:9569807 ;     AAGGATGCTGTCAGCTGCCTTCTTG       HiG6 CAPS markers     C/T     GO7_R     using HinfI     TGGATACAGGTGGCCTATGGTGTC       n.a. not     applicable     Image: Construct of the second sec  | oAS806     | using XmnI                | scaffold_7:9374725;  | CGTTGCAGAGAGCTTTAATAGAATTTC     |
| oAS807     using XmnI     GTCGCTTGCCACGATTGGGTAATA       HiG6 CAPS markers     HiG6 CAPS markers       G07_F     using HinfI     scaffold_7:9569807 ;       HiG6 CAPS markers     C/T       G07_R     using HinfI       n.a. not     applicable   |            | HiG5 CAPS markers         | A/C                  |                                 |
| HiG6 CAPS markers         G07_F       using Hinfl       scaffold_7:9569807 ;       AAGGATGCTGTCAGCTGCCTTCTTG         HiG6 CAPS markers       C/T       GO7_R       using Hinfl       TGGATACAGGTGGCCTATGGTGTC         n.a. not       applicable       Fight State       Fight State       Fight State   | oAS807     | using XmnI                |                      | GTCGCTTGCCACGATTGGGTAATA        |
| G07_F       using Hinfl       scaffold_7:9569807;       AAGGATGCTGTCAGCTGCCTTCTTG         HiG6 CAPS markers       C/T         G07_R       using Hinfl       TGGATACAGGTGGCCTATGGTGTC         n.a. not       applicable  |            | HiG6 CAPS markers         |                      |                                 |
| HiG6 CAPS markers     C/T       G07_R     using Hinf1     TGGATACAGGTGGCCTATGGTGTC       n.a. not     applicable  | G07_F      | using Hinfl               | scaffold 7:9569807 : | AAGGATGCTGTCAGCTGCCTTCTTG       |
| G07_R     using Hinfl     TGGATACAGGTGGCCTATGGTGTC       n.a. not     applicable  |            | HiG6 CAPS markers         | С/Т                  |                                 |
| n.a. not<br>applicable  | G07 R      | using Hinfl               | <i>C</i> , <i>1</i>  | TGGATACAGGTGGCCTATGGTGTC        |
| applicable  | n.a. not   | <u>0</u> y-               |                      |                                 |
|   | applicable |                           |                      |                                 |

### **Supplementary Discussion**

#### Genetic incompatibilities in the genus Capsella.

The genetic incompatibility in the  $Cr1504 \ge Cg926$  RIL population suggested that the presence within the same cells of RPP5 and NPR1<sup>go</sup> induces an autoimmune response. If true, any crosses combining a functional RPP5 protein with NPR1<sup>go</sup> should recreate the stunted growth phenotype. We tested this hypothesis by performing random crosses between seven *C. rubella* and nine *C. grandiflora* accessions (Supplementary Fig. 7). A phenotype similar to the *Cr1504* x *Cg926* incompatible hybrids was segregating in 13 of the resulting F<sub>2</sub> populations.

The RPP5 alleles from three of the C. rubella accessions used for the crosses (Cr4.23, *Cr86IT1-C* and *Cr1377/5*) belong to different haplotype groups than *Cr1504RPP5* (Supplementary Fig. 5b), while no RPP5 could be detected in a fourth C. rubella accession (Cr 1GR1-TS1). Among the C. grandiflora x C. rubella F<sub>2</sub> progenies descending from these C. rubella accessions, we did not observe any correlation between the RPP5 genotype (as determined by a tightly linked molecular marker HiG2 to be able to assess the genotype at the RPP5 locus also in progeny of Cr 1GR1-TS1) and the stunted growth. Similarly, no correlation was observed in the  $F_2$  population descending from Cr22.5 in cross 21, whose *RPP5* allele belongs to the same haplotype group as Cr1504RPP5. We note that the absence of incompatible phenotypes in cross 12, also involving Cr22.5, and in cross 4 is not informative, as the C. grandiflora parental plants of these crosses did not pass on an NPR1<sup>go</sup> allele to the respective F1 plants. This is due to the C. grandiflora accession Cg926 being heterozygous for NPR1<sup>go</sup>/NPR1<sup>rub</sup> (see also Fig. 3a). In fact, a robust correlation between incompatible phenotype and *RPP5* genotype was only seen in F<sub>2</sub>s descending from crosses between Cr1504 and diverse C. grandiflora individuals. These results indicate that only a specific *RPP5* haplotype is incompatible with the *NPR1<sup>go</sup>* alleles. It is therefore likely that after the divergence of C. rubella from C. grandiflora, a novel mutation in Cr1504RPP5 has rendered it incompatible with NPR1<sup>go</sup>. This hypothesis is supported by the observation that no correlation between stunted growth phenotypes and RPP5/NPR1 genotypes was observed within natural C. grandiflora populations.

In addition, modifier alleles of the *NPR1/RPP5* incompatibility appear to be segregating in *C*. *grandiflora*. This is based on the observation that the strength and frequency of the stunted growth varies between the different  $F_{28}$  descending from *Cr1504*. For example, although the same *NPR1<sup>go</sup>* and *RPP5* haplotype are segregating in the crosses 6 and 7, only 10 % of the

F<sub>2</sub>s show an incompatible phenotype in cross 7 versus 30% in cross 6 (Supplementary Fig. 6A,S7), and the phenotypic severity is reduced in the cross 7 relative to the cross 6 F<sub>2</sub> progenies (Supplementary Fig. 7B). Since *Cr1504* is highly inbred, this indicates that modifiers affecting the penetrance and expressivity of the genetic incompatibility are segregating within *C. grandiflora*.

Importantly, we observed that NPR1<sup>go</sup> haplotypes are also associated with genetic incompatibilities that do not involve RPP5 (Supplementary Fig. 7; crosses 3 and 9). In the progeny of cross 9, all plants homozygous for the NPR1<sup>go</sup> allele showed the incompatible phenotype, while none of the plants homozygous for NPR1<sup>rub</sup> did so, yet there was no difference in the RPP5 genotype frequency between plants with incompatible and compatible phenotypes. Similarly, in the F<sub>2</sub> of cross 3, in which no *RPP5* presence could be detected, all but one of the 39 incompatible hybrids had at least one copy of the NPR1<sup>go</sup> allele, and NPR1<sup>go</sup> homozygotes were underrepresented amongst compatible plants. Individual NPR1<sup>rub</sup> homozygotes amongst the incompatible plants were also observed in two of the crosses involving Cr1504, which is likely due to mis-scoring of the phenotype or to unrelated deleterious mutations from the C. grandiflora parent that became homozygous in the  $F_2$ . Given also the molecular evidence for a constitutive auto-immune response in the incompatible plants in the F<sub>2</sub> from cross 3 (Supplementary Fig. 8a,b), we consider it highly likely that this incompatibility is also caused by the NPR1<sup>go</sup> allele. In the F<sub>2</sub> of cross 10, the stunted growth phenotype co-segregated with the NPR1<sup>rub</sup> allele, but not with the RPP5 genotype (Supplementary Fig. 7). While suggestive of an involvement of NPR1<sup>rub</sup> in the incompatibility, several incompatible plants were homozygous for NPR1<sup>go</sup>, and further experiments will be needed to test a causal role for NPR1<sup>rub</sup>. In any case, these results indicate that alleles at additional loci are incompatible with NPR1<sup>go</sup> and possibly NPR1<sup>rub</sup>; these alleles are likely to segregate in C. grandiflora, as the incompatibilities were only observed in some, but not all crosses involving a given C. rubella accession (compare for example crosses 3, 20 and 24). Together, these results support the notion that the two NPR1 haplotype groups with their strong sequence divergence resulting from long-term balancing selection facilitate the establishment of genetic incompatibilities by mutations to interacting loci. If any such strongly divergent haplotypes were sorted into derived populations in a mutually exclusive manner, for example following genetic bottlenecks such as during independent transitions to selfing from an ancestral outbreeding species, the first step to the formation of a gene-flow barrier would already have been achieved, and only one further

mutation to an interacting locus in one of the derived populations would be required for the establishment of a BDMI.

### **Supplementary Reference**

1. Sicard, A. *et al.* Genetics, evolution, and adaptive significance of the selfing syndrome in the genus Capsella. *Plant Cell* **23**, 3156-3171 (2011).